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Urinary Renin-Angiotensin Markers in Polycystic Kidney Disease

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Abstract

In autosomal dominant polycystic kidney disease (ADPKD) activation of the renin-angiotensin aldosterone system (RAAS) may contribute to hypertension and disease progression. Although previous studies focused on circulating RAAS-components, preliminary evidence suggests ADPKD may increase urinary RAAS-components. Therefore, our aim was to analyze circulating and urinary RAAS-components in ADPKD. We cross-sectionally compared 60 patients with ADPKD to 57 patients with non-ADPKD chronic kidney disease (CKD). The two groups were matched by gender, estimated glomerular filtration rate (eGFR), blood pressure, and RAAS-inhibitor use. Despite similar plasma levels of angiotensinogen and renin, urinary angiotensinogen and renin excretion were 5- to 6-fold higher in ADPKD ($P<0.001$). These differences persisted when adjusting for group differences, and were present regardless of RAAS-inhibitor use. In multivariable analyses, ADPKD, albuminuria, and the respective plasma concentrations were independent predictors for urinary angiotensinogen and renin excretion. In ADPKD, both plasma and urinary renin correlated negatively with eGFR. Total kidney volume correlated with plasma renin and albuminuria, but not with urinary renin or angiotensinogen excretions. Albuminuria correlated positively with urinary angiotensinogen and renin excretions in ADPKD and CKD. In three ADPKD patients who underwent nephrectomy, the concentrations of albumin and angiotensinogen were highest in plasma followed by cyst fluid and urine; urinary renin concentrations were higher than cyst fluid. In conclusion, this study shows that, despite similar circulating RAAS-component levels, higher urinary excretions of angiotensinogen and renin are a unique feature of ADPKD. Future studies should address the underlying mechanism and whether this may contribute to hypertension or disease progression in ADPKD.

51 **Introduction**

52 Hypertension develops early in autosomal dominant polycystic kidney disease (ADPKD),
53 usually occurring before a reduction in glomerular filtration rate (GFR) with an average age
54 of onset of 30 years (5, 9). Increased activity of the renin-angiotensin-aldosterone system
55 (RAAS) has been implicated in the pathogenesis of hypertension in ADPKD. One hypothesis
56 is that cyst expansion results in areas of local renal ischemia which increases renin release (3,
57 5). In addition, renin has also been suggested to be produced by the epithelial cells lining the
58 cysts and active renin can be found within the cyst fluid (12, 36). However, measurement of
59 plasma renin and aldosterone in patients with ADPKD yielded equivocal results (**Table 1**).
60 Several studies found that plasma renin and aldosterone concentrations were not higher in
61 hypertensive ADPKD patients when compared to controls, even during specific interventions
62 (low or high sodium diet, ACE-inhibition, angiotensin II infusion) (1, 7, 21, 29, 37, 40).
63 Different control groups were used for these studies, including normotensive ADPKD
64 patients, normotensive siblings without ADPKD, patients with essential hypertension, or
65 healthy volunteers (**Table 1**). The observation that plasma renin activity was not consistently
66 higher in hypertensive ADPKD patients is notable, because this is contrary to what would be
67 expected if cysts caused local renal ischemia (3, 5). Therefore, to further address the role of
68 the RAAS in ADPKD, it may be informative to analyze RAAS-components in urine, as
69 urinary angiotensinogen and renin have previously been used as markers of the intra-renal
70 renin-angiotensin system (32). Emerging data suggest that filtered or locally produced
71 RAAS-components may activate this intrarenal renin-angiotensin system and thereby
72 contribute to hypertension (11). Two recent studies reported higher urinary angiotensinogen
73 concentrations in hypertensive ADPKD patients (15, 25). However, despite its postulated
74 central role in the pathogenesis of hypertension, urinary renin has never been measured in
75 patients with ADPKD. We recently showed that it is important to measure urinary renin with

standardized assays, because commercial assays may produce ≥ 10 -fold higher results (31). Therefore, here, we measured urinary renin in patients with ADPKD using a validated renin immunoradiometric assay and an in-house enzyme kinetic assay. In addition, we also measured multiple other RAAS-components in plasma and urine, including plasma renin and aldosterone, and urine angiotensinogen, prorenin, and aldosterone. As a comparator, and for the first time, we used matched patients with non-ADPKD chronic kidney disease (CKD) to address whether the type of kidney injury affects the RAAS differently.

Materials and Methods

Patients

Patients with ADPKD were recruited from one of the centers (Erasmus Medical Center, Rotterdam, The Netherlands) participating in a national ADPKD consortium (DIPAK study, with inclusion criteria CKD stage 3 at entry into the study and age ≤ 60 years) (23). Patients were matched to non-ADPKD CKD patients (referred to hereafter as ‘CKD’) from the PREVEND cohort (University Medical Center Groningen) (18). The Medical Ethics Committees of the Erasmus Medical Center and University Medical Center Groningen approved the studies (MEC-2012-313 and METC-90/01/022). Patients with ADPKD and CKD were individually matched for gender, eGFR (using the Modification of Diet in Renal Disease equation (19)), use of RAAS-inhibitors (defined as the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers) and blood pressure (difference in systolic blood pressure ≤ 5 mmHg). Patients with a history of diabetes mellitus, or those using insulin or oral glucose lowering drugs were excluded, because diabetes mellitus may activate the intrarenal renin-angiotensin system (38). From three ADPKD patients who underwent elective nephrectomy (not part of the DIPAK cohort), we collected plasma, cyst fluid, and urine samples. For this part of the study, a separate approval from the Medical Ethics Committee of the University Medical Center Groningen was obtained (METC 2014.396).

Data Collection

Detailed description of data collection for both the DIPAK and PREVEND studies has been described elsewhere (18, 23). Briefly, participants of both studies collected 24-hour urine and visited the outpatient clinic for blood sampling and blood pressure measurements using an automatic oscillometric device. Hypertension was defined as a blood pressure $> 140/90$ mmHg or the use of anti-hypertensive medication. Participants were instructed to store urine

at 4°C during collection. Upon arrival in the university medical center, blood and urine samples were immediately stored at -80°C until further use. We have previously shown that in 24-hour urine, prorenin is not converted into renin prior to freezing (38). To determine adequate 24-hour urine collection, we calculated the expected 99% quantile of creatinine excretion based on previously defined criteria (10). Patients who exceeded the expected range were excluded (8 patients with ADPKD and 1 patient with CKD). ADPKD patients underwent magnetic resonance imaging to determine total kidney volume (23). The World Health Organization defined daily dose (DDD) was used to calculate daily RAAS-inhibitor use.

Measurements

Plasma and urine samples from the DIPAK and PREVEND cohorts were measured simultaneously. Renin in plasma was measured with a commercially available immunoradiometric kit (Renin III; Cisbio, Gif-sur-Yvette, France), making use of an active site-directed radiolabeled antibody (4). Total plasma renin was determined simultaneously using the same kit after the induction of a conformational change in the prorenin molecule with aliskiren (10 µmol/l for 48 hours at 4°C), which enabled its recognition by the active site-directed radiolabeled antibodies applied in the Cisbio kit (2). The detection limit of this assay is 1 pg/ml with intra- and inter-assay coefficients of variation (CVs) of 2.4% and 7.2%. Urinary renin and urinary total renin (after prorenin activation with trypsin) were measured with an in-house enzyme kinetic assay (EKA) (16). This measurement involves the incubation of the urine sample with excess sheep angiotensinogen and angiotensinase inhibitors and the subsequent detection of the generated Ang I by radioimmunoassay. The detection limit of the EKA is 0.05 ng Ang I/ml per hour with intra- and inter-assay CVs of 2.9 and 12.6%. Ang I-generating activities were converted to renin concentrations based on

the fact that 1 ng Ang I/ml per hour corresponds with 2.6 pg human renin/ml (16). Prorenin was determined by subtraction of renin from total renin. Angiotensinogen in plasma and urine was measured as the maximum quantity of Ang I that was generated during incubation with excess recombinant renin (6). The detection limit of this assay is 0.50 pmol/ml with intra- and inter-assay CVs of 4 and 10%. Aldosterone was measured by solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California, USA), with a detection limit of 25 pg/ml with intra- and inter-assay CVs of 3.3 and 8.4% (14).

Statistical Analyses

Results are expressed as mean and standard deviation or median and range, as appropriate. Data were logarithmically transformed before analysis in case of non-normal distribution. Levels that were below the detection limit were considered to be half the detection limit to allow for statistical analysis (8). Analysis of variance (ANOVA) was used for group comparison (using log-transformed data as appropriate). Further analysis was performed using analysis of covariance (ANCOVA) to adjust for covariates. To analyze which parameters independently predicted urinary angiotensinogen or renin excretion, we performed multivariable linear regression. Finally, the Pearson correlation coefficient was analyzed for selected variables. A P -value < 0.05 was considered statistically significant. Statistical analyses were performed with SPSS (version 21, IBM).

Results

ADPKD Increases Urinary Angiotensinogen and Renin Excretion

Table 2 shows the baseline characteristics and RAAS-component measurements for the ADPKD and CKD groups. Patients with ADPKD were younger (47 vs. 68 years), taller (175 vs. 169 cm), and used more RAAS-inhibitors. While plasma levels of angiotensinogen, renin, and aldosterone were similar between the two groups, 24-hour urine volume, and urinary albumin, angiotensinogen, renin, and aldosterone excretions were significantly higher in patients with ADPKD ($P < 0.05$ for all). Similarly, when expressed as ratio with creatinine, urinary angiotensinogen and renin were also significantly higher in ADPKD (urinary angiotensinogen 14.6 vs. 3.3 pmol/mol creatinine, urinary renin 204 vs. 44 pg/mol creatinine, $p < 0.01$ for both). Because of the group differences in age, height, DDD, and albuminuria, we also performed a second analysis adjusting for these factors (**Table 2**). This analysis showed that urinary angiotensinogen and renin excretion were still significantly higher in ADPKD than CKD ($P < 0.001$ for both). In addition, a subanalysis was performed in patients ($n = 17$ vs. 11) with similar age, gender, height and a similar degree albuminuria, which also showed that urinary angiotensinogen (286.2 vs. 38.7 pmol/day) and renin (1874 vs. 398.6 pg/day) excretions were significantly higher in ADPKD compared to CKD ($P < 0.05$ for both).

Effects of RAAS-Inhibitors

Because the use of RAAS-inhibitors increases plasma renin, this may also increase urinary renin. Therefore, we also report the plasma and urinary RAAS-components in patients with and without RAAS-inhibitor use (**Figure 1**). Plasma renin was indeed significantly higher in both ADPKD and CKD patients using RAAS-inhibitors. In patients *without* RAAS-inhibitors, plasma renin was significantly *lower* in ADPKD than in CKD. Despite these

differences in plasma renin, urinary renin excretion was consistently higher in the patients with ADPKD than in the patients with CKD regardless of RAAS-inhibitor use (**Figure 1**). Urinary angiotensinogen excretion was significantly higher only in patients with ADPKD and RAAS-inhibitor use, but this may be a power issue, as few patients were without RAAS-inhibitors.

Predictors of Urinary Angiotensinogen and Renin Excretion

Two multivariable linear regression analyses were performed to analyze which factors independently predict urinary angiotensinogen or urinary renin excretion (**Table 3**). In the model we included the presence of ADPKD, eGFR, age, DDD, plasma concentrations of angiotensinogen and renin, and urinary sodium and albumin excretion. For urinary angiotensinogen excretion, ADPKD, eGFR, ~~plasma angiotensinogen~~, and albuminuria were identified as independent predictors. For urinary renin excretion, ADPKD, plasma renin, and albuminuria were identified as independent predictors. When the analyses were restricted to patients with ADPKD, only albuminuria predicted urinary angiotensinogen excretion, and only plasma renin predicted urinary renin excretion (data not shown).

Correlations with Total Kidney Volume and Kidney Function

Within the ADPKD group, we analyzed whether circulating or urinary RAAS-components correlated with total kidney volume and kidney function (eGFR). A higher total kidney volume correlated with higher plasma renin, and more albuminuria, but not with urinary angiotensinogen or renin excretion (**Figure 2A**). Both higher plasma renin and higher urinary renin excretion correlated with lower eGFR (**Figure 2B**). To analyze the possible mechanism of urinary angiotensinogen and renin excretion, we analyzed in the ADPKD and CKD groups whether these two urinary RAAS-components correlated with albuminuria. Indeed, both in

patients with ADPKD and CKD, a higher degree of albuminuria correlated with higher urinary angiotensinogen or renin excretion, although the strength of this correlation was modest (**Figure 2C**). For urinary renin excretion, this correlation was of borderline significance in patients with ADPKD ($P = 0.06$).

Comparison of Concentrations in Plasma, Cyst Fluid, and Urine

In three patients with ADPKD who underwent nephrectomy, we measured albumin, angiotensinogen, prorenin, and renin in plasma, cyst fluid (average concentration of five cysts) and urine. For all four parameters, the concentrations were highest in plasma followed by cyst fluid and urine (**Figure 3**). Of interest, urinary concentrations were lower than cyst concentrations except for renin. Urinary prorenin concentrations were close to or below the detection limit.

Discussion

This study reveals a unique feature of patients with ADPKD, namely a consistently higher urinary excretion of angiotensinogen and renin compared to patients with CKD. Urinary angiotensinogen and renin excretions were 5- to 6-fold higher in patients with ADPKD than in patients with CKD, who were matched by eGFR, blood pressure, and RAAS-inhibitor use (**Table 2**). ADPKD remained a significant predictor for urinary angiotensinogen and renin excretion in adjusted and in multivariable analyses (**Tables 2 and 3**), and regardless of RAAS-inhibitor use (**Figure 1**). Recent studies found higher urinary angiotensinogen to creatinine ratios in normotensive ADPKD patients compared to healthy controls (17), or higher levels within ADPKD patients in the presence of hypertension (15) or reduced kidney function (25). The magnitude of the urinary angiotensinogen levels reported in these previous studies are comparable to our data. Our study is the first to analyze urinary renin and to use patients with CKD as control group (**Table 1**). Although our cross-sectional study cannot give definitive answers, our data give directions on the possible mechanisms and potential clinical implications of the increased urinary excretions.

In principle, urinary angiotensinogen and renin excretion can increase in ADPKD because of (1) damage to the glomerular filtration barrier, (2) reduced proximal tubular reabsorption, (3) enhanced tubular secretion by intact nephrons, (4) differences in degradation, or (5) ectopic production by cyst-lining epithelial cells.

When evaluating the first two possibilities, it is important to correct for differences in the plasma levels. Although the correlation between total kidney volume and plasma renin in ADPKD patients indeed suggests that renal ischemia by cysts can increase plasma renin (**Figure 2A**), patients with ADPKD in general do not have higher plasma renin

245 concentrations than patients with CKD (**Table 2**). In fact, patients with ADPKD *without*
 246 RAAS-inhibitors had significantly *lower* plasma renin concentrations than patients with CKD
 247 (**Figure 1**). Thus, the higher urinary excretions of angiotensinogen and renin in ADPKD are
 248 not simply the consequence of elevated plasma RAAS concentrations exposed to the same
 249 degree of filtration and reabsorption as in CKD patients. In addition, increase of cysts (i.e.
 250 total kidney volume) did not correlate with increased excretion of urinary angiotensinogen or
 251 renin. Next, it is important to emphasize that ADPKD is a primarily a tubular disorder that is
 252 less likely to damage the glomerular filtration barrier (24). Indeed, previous studies have
 253 attributed albuminuria in animal models of ADPKD to disturbed endocytosis of albumin in
 254 the proximal tubule (24, 41). In these studies, immunohistochemistry showed less expression
 255 of the chloride channel CIC-5 and megalin, which are both involved in the reabsorption of
 256 low-molecular weight proteins. Because albumin, angiotensinogen, and renin are all
 257 reabsorbed by a megalin-dependent pathway, a proximal tubular disorder should by
 258 definition result in higher urinary angiotensinogen and renin excretion, even in the face of
 259 identical or lower plasma RAAS-component levels (28, 30). In agreement with this concept,
 260 we recently showed that patients with Dent's disease (who lack CIC-5) displayed a 20-40-
 261 fold rise in urinary angiotensinogen and renin levels, although their plasma RAAS levels
 262 were in the normal range (30). Similarly, other urinary markers of proximal tubule damage,
 263 such as fetuin-A and β 2-microglobulin, are increased in ADPKD (22, 27). The ADPKD
 264 component that independently predicted urinary angiotensinogen and renin excretion in our
 265 multivariable regression analysis therefore possibly reflects a difference in tubular
 266 reabsorption. We showed that albuminuria correlated with total kidney volume (**Figure 2A**),
 267 as was shown previously (34). In addition to ADPKD, albuminuria also independently
 268 predicted urinary renin and angiotensinogen excretion. This also suggests that the urinary
 269 excretions of albumin, angiotensinogen, and renin was at least in part due to similar

mechanisms, and argues against selective tubular secretion of RAAS-components. This leaves the issue of altered degradation. Reduced reabsorption would also be expected to result in elevated levels of RAAS-component degrading enzymes, leading to enhanced degradation. Yet, higher levels of urinary RAAS-components were found in ADPKD. Combined with data from previous studies showing no evidence for urinary degradation of renin or prorenin (38), and identifying all urinary angiotensinogen as intact (and not cleaved) (39), it appears that reduced degradation does not underlie the increased urinary excretion of angiotensinogen and renin in ADPKD.

The possibility of ectopic RAAS-component production by cyst-lining epithelial cells has been suggested by several investigators (20, 35). Although we cannot entirely exclude this possibility, such local production should have resulted in angiotensinogen and renin concentrations in cyst fluid that would have been at least comparable to, if not far above, their plasma concentrations. Remarkably, this was not the case (**Figure 3**). In fact, relative to albumin, the concentrations of angiotensinogen, renin and prorenin were lower in cyst fluid (**Figure 3**). In other words, when using cyst albumin concentrations as a measure of blood-derived proteins, cyst RAAS-component concentrations can be entirely explained on the basis of leakage from blood plasma. Of interest, the urinary concentrations of albumin, angiotensinogen, and prorenin were lower than their concentrations in cyst fluid. This most likely reflects further dilution and/or tubular reabsorption. Surprisingly, this was not the case for renin: its concentrations in cyst fluid and urine were similar (**Figure 3**). Moreover, the correlation between albumin and renin excretion, although modest, showed a different pattern in ADPKD than in CKD (**Figure 2C**). Taken together, these data suggest that ADPKD affects the urinary excretion of renin differently than of albumin, angiotensinogen and prorenin. This difference between renin and prorenin excretion (resulting in urinary prorenin

levels that are virtually undetectable), has been observed before (30, 38). Of note, we did not measure the sodium concentration in cyst fluid; previously, more active renin was found in so-called “gradient cysts” in which the sodium concentration is low (36).

Many aspects of the intra-renal renin-angiotensin system remain unclear, although some groups have suggested that angiotensinogen and renin in tubular fluid may lead to high tubular angiotensin II levels (11, 20). Such high local angiotensin II levels could promote renal sodium retention, hypertension, kidney damage, or even cystogenesis (20). Of interest in this regard is a recent report where targeting the intra-renal renin-angiotensin system reduced cyst growth in an animal model of polycystic kidney disease (33). Based on our cross-sectional data, we cannot conclude whether the higher urinary angiotensinogen and renin excretions are a cause or a consequence of the kidney damage in ADPKD. In other words, it is unclear if higher urinary angiotensinogen and renin excretion should be considered as a damage marker or as a potential contributor to kidney damage. Given the experimental link between the intra-renal renin-angiotensin system and cystogenesis, this deserves further study.

A number of limitations of this study should be mentioned. First, RAAS-inhibitor use could have influenced our results. Ideally, urinary RAAS-components should be measured before and after starting a RAAS-inhibitor. Previous studies have shown a decrease of urinary angiotensinogen and no effects on urinary renin after initiation of an angiotensin receptor blocker in patients with chronic kidney disease (26, 42). Therefore, if anything, our ADPKD group would be expected to have lower urinary angiotensinogen excretion, which was not the case. Second, despite matching of ADPKD with CKD patients by the most relevant parameters (eGFR, blood pressure, RAAS-inhibitors), several differences remained. The

difference in age was inevitable, as eGFR decline occurs much earlier in ADPKD than in other forms of CKD. We addressed these differences by using an adjusted analysis, multivariable analyses, and a subanalysis (**Table 2, Table 3**). Finally, although significant, the strength of the correlations observed in this study were modest, suggesting high inter-individual variability or a multifactorial origin.

In conclusion, ADPKD uniquely increases urinary angiotensinogen and renin excretion despite their circulating levels being comparable to those in CKD. We believe these findings warrant further analysis in mechanistic or intervention studies.

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346

347 **Disclosures**

348 None to declare.

349

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Table 1: Comparison of studies measuring RAAS-components in patients with ADPKD

Study	Cases	CKD stage	Controls	Numbers	Measurement(s)	Difference
Valvo (37)	ADPKD + HT	1-3	ADPKD + NT	20 vs. 12	PRA	↔
Bell (3)	ADPKD + HT	1-2	ADPKD + NT	9 vs. 7	PRA during low/ high Na ⁺ diet + ACEi	↔ but ↑ during ACEi + high Na ⁺ diet
Chapman (5)	ADPKD	1	Essential HT + healthy controls	14 + 11 vs. 9 + 13	PRA and aldo during ACEi	PRA and aldo ↑ in ADPKD + HT
Harrap (13)	ADPKD	1	Siblings	19 vs. 20	PRA and aldo	PRA and aldo ↑
Watson (40)	ADPKD	1-2	Siblings	13 vs. 10	PRA	↔
Barrett (1)	ADPKD	1-2	Siblings	21 vs. 12	PRA and aldo during low/high Na ⁺ diet, ACEi, Ang II infusion	↔
Martinez-Vea (21)	ADPKD + HT	2-3	Essential HT	20 vs. 20	PRA, aldosterone, ANP, Ang II	↔
Ramunni (29)	ADPKD + HT	1-2	ADPKD + NT	17 vs. 17	PRA	↔
Doulton (7)	ADPKD + HT	1-2	Essential HT	11 vs. 8	PRA during low/high Na ⁺ diet and ACEi	↔
Kurultak (17)	ADPKD	1	Healthy controls	20 vs. 20	Urinary AGT	↔
Kocyigit (15)	ADPKD + HT	1-2	ADPKD + NT, healthy controls	43 vs. 41 + 40	Plasma and urinary AGT	Urinary AGT ↑
Park (25)	ADPKD	1-5	None	186	Plasma renin+ aldo, urinary AGT	Correlation urinary AGT with eGFR, TKV, BP
Present study	ADPKD	3	CKD	69 vs. 58	Plasma + urinary AGT, renin, aldo	Urinary AGT + renin ↑

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ACEi, angiotensin converting enzyme inhibitor; AGT, angiotensinogen; aldo, aldosterone; Ang II, angiotensin II; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HT, hypertension; NT, normotension; PRA, plasma renin activity; BP, blood pressure.

509 **Table 2:** Patient characteristics and renin-angiotensin-aldosterone system measurements.

Category	Parameter	ADPKD (n = 60)	CKD (n = 57)	P- value*	P- value**
Clinical data	Age, years	47 ± 8	68 ± 8	< 0.001	
	Male gender, n (%)	25 (42)	25 (44)	N.T.¶	
	Height, cm	175 ± 10	169 ± 9	0.001	
	Weight, kg	81.6 ± 17.0	81.2 ± 11.9	0.9	
	Hypertension, n (%)†	56 (93)	49 (86)	N.T.	
	SBP, mmHg	131 ± 14	134 ± 14	N.T.	
	DBP, mmHg	79 ± 9	76 ± 7	N.T.	
	RAAS-inhibitors, n (%)	50 (83)	44 (77)	N.T.	
	DDD RAAS-inhibitors, n	1.9 ± 1.5	1.1 ± 0.9	< 0.001	
	DDD RAAS-inhibitors, n	1.9 ± 1.5	1.1 ± 0.9	< 0.001	
Plasma	Creatinine, mg/dL	1.5 ± 0.4	1.4 ± 0.4	0.6	
	eGFR, ml/min per 1.73 m ²	48 ± 11	46 ± 9	N.T.	
	Angiotensinogen, pmol/mL	1599 (313–8067)	1455 (474–3567)	0.3	
	Renin, pg/mL	81.3 (9.6–950.0)	68.3 (14.8–464.5)	0.3	
	Aldosterone, pg/mL	121.7 (16.5–470.1)	105.9 (16.6–546.4)	0.2	
Urine	Volume, mL/day	2233 (800–6500)	1652 (530–3140)	< 0.001	0.2
	Creatinine, mmol/day	13.3 (5.2–21.2)	10.9 (6.0–18.2)	< 0.001	0.8
	Albumin, mg/day	40.0 (3.1–266.4)	26.7 (3.4–293.2)	0.05	-
	Sodium, mmol/day	150 (40 – 354)	142 (60 – 371)	0.5	
	Angiotensinogen, pmol/day	194.4 (3.5–3384.0)	36.0 (2.3–1070)	< 0.001	< 0.001
	Renin, pg/day	2717 (375.7–69248.0)	485.5 (154.7–2293.0)	< 0.001	< 0.001
	Aldosterone, µg/day	4.6 (0.9–32.8)	3.5 (1.0–18.0)	0.02	0.2

510 **Footnotes:**

511 * Using analysis of variance (ANOVA) with log-transformed data as appropriate

512 ** Using analysis of covariance (ANCOVA) with log-transformed data as appropriate

513 and adjustments for age, height, defined daily dose, and albuminuria

514 ¶ N.T., not tested (matching criteria). † Defined by use of antihypertensive drugs.

515

516 **Abbreviations:** ADPKD, autosomal dominant polycystic kidney disease; CKD, chronic
517 kidney disease; DBP, diastolic blood pressure; DDD, defined daily dose; eGFR, estimated
518 glomerular filtration rate; SBP, systolic blood pressure.

519

Table 3: Multivariable analysis of factors predicting urinary angiotensinogen and renin excretion.

Variable	Urinary angiotensinogen excretion		Urinary renin excretion	
	β	<i>P</i> -value	β	<i>P</i> -value
Presence of ADPKD	9.6 (3.7 – 24.5)	< 0.001	4.9 (2.6 – 9.0)	< 0.001
eGFR, ml/min/1.73 m ²	0.96 (0.94 – 0.99)	0.002	0.99 (0.98 – 1.01)	0.3
Age, years	1.0 (0.9 – 1.1)	0.2	0.99 (0.97 – 1.02)	0.6
DDD RAAS-inhibitors, n	0.8 (0.7 – 1.0)	0.1	0.9 (0.8 – 1.1)	0.2
Plasma renin, pg/mL	0.5 (0.3 – 1.0)	0.05	1.9 (1.2 – 2.9)	0.007
Plasma AGT, pg/mL	3.0 (0.9 – 10.7)	0.08	1.1 (0.5 – 2.4)	0.9
Urinary sodium, mmol/day	1.6 (0.4 – 7.1)	0.5	1.0 (0.4 – 2.8)	0.9
Albuminuria, mg/day	6.2 (3.6 – 10.7)	< 0.001	1.6 (1.1 – 2.3)	0.01

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; AGT, angiotensinogen; DDD, defined daily dose; eGFR, estimated glomerular filtration rate; RAAS, renin-angiotensin-aldosterone system

Legends to Figures

Figure 1: Plasma concentrations and urinary excretions of angiotensinogen and renin in patients with ADPKD or CKD and with or without RAAS-inhibitors

Box-and-whisker plots of plasma concentrations and urinary excretions of renin and angiotensinogen for ADPKD (light grey) and CKD (dark grey). Groups were subdivided into those with (+) and without (-) use of RAAS-inhibitors (RAASi). Of the 60 patients with ADPKD, 10 patients did not use RAASi; of the 57 CKD patients, 13 patients did not use RAASi. Boxes show the median, interquartile range and range. ANOVA was used for comparison with * $P < 0.05$.

Figure 2: Correlations of RAAS-components with total kidney volume and kidney function

Pearson correlation coefficients were calculated using log-transformed data. Height-adjusted total kidney volume was available in 51 patients with ADPKD. Correlations between urinary albumin, angiotensinogen, and renin excretion are shown both for patients with ADPKD and CKD.

Figure 3: Concentrations of Albumin and RAAS-components in plasma, cyst fluid, and urine

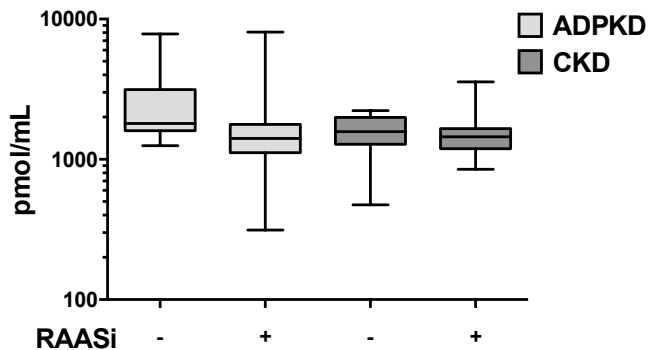
With plasma values set to 1, this figure shows the relative mean concentrations of albumin, angiotensinogen (AGT), prorenin, and renin in plasma (P), cyst fluid (C) and urine (U). The actual mean plasma concentrations were 42 g/L, 1814 pmol/L, 573.8 pg/mL, and 81.3 pg/mL, respectively. Measurements were performed in three ADPKD patients, who underwent elective nephrectomy to create space for kidney transplantation (45 and 52 year-old males

553 with eGFRs of 12 and 9 mL/min/1.73 m², respectively) or because of mechanical discomfort

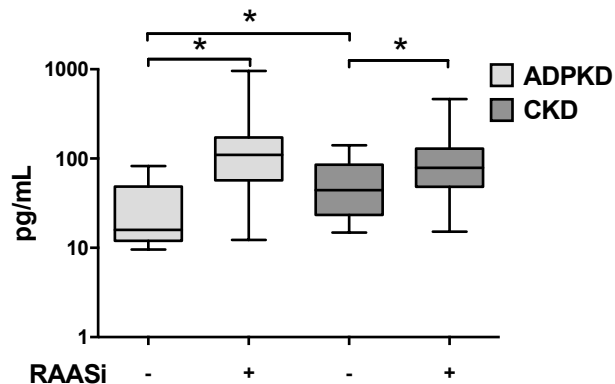
554 (71-year-old female, eGFR 18 mL/min/1.73 m²).

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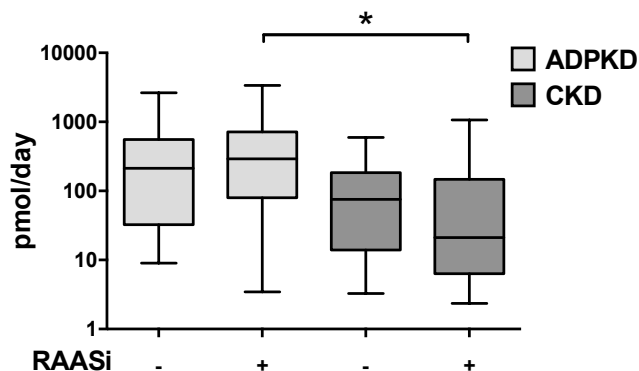
Plasma angiotensinogen



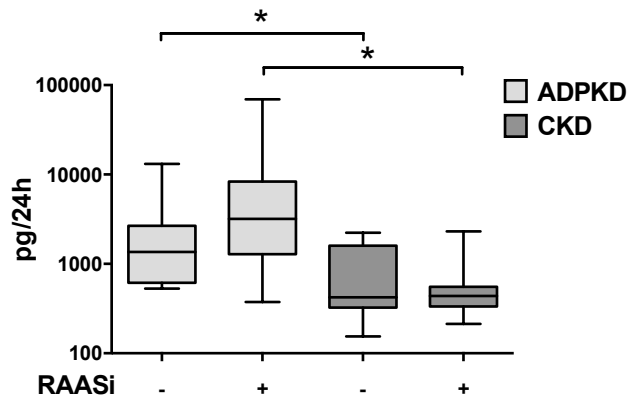
Plasma renin



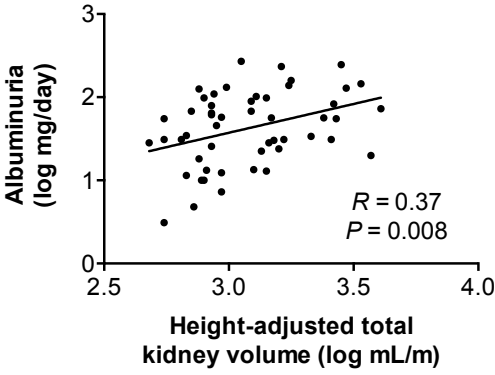
Urinary angiotensinogen excretion



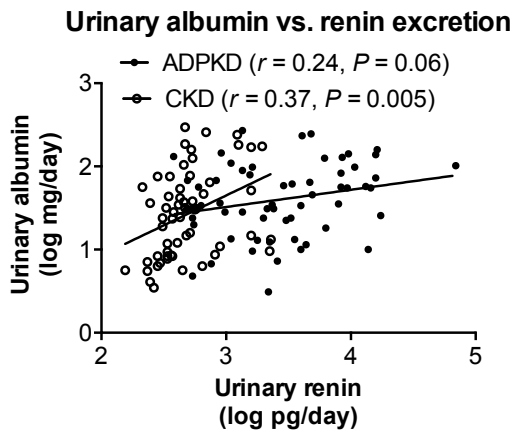
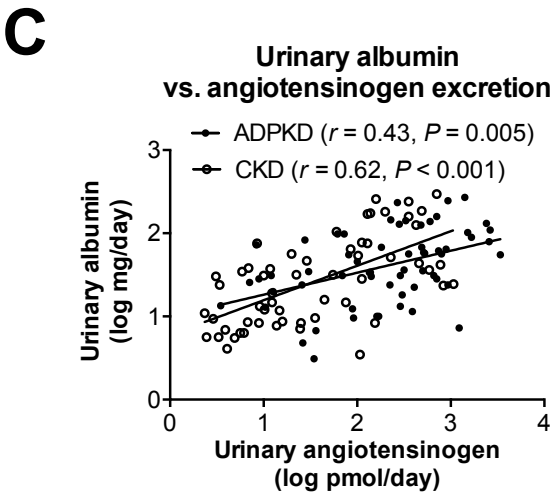
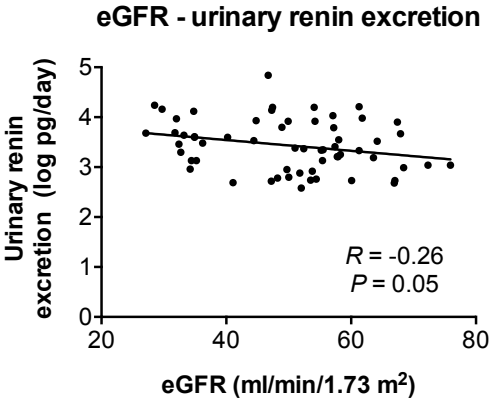
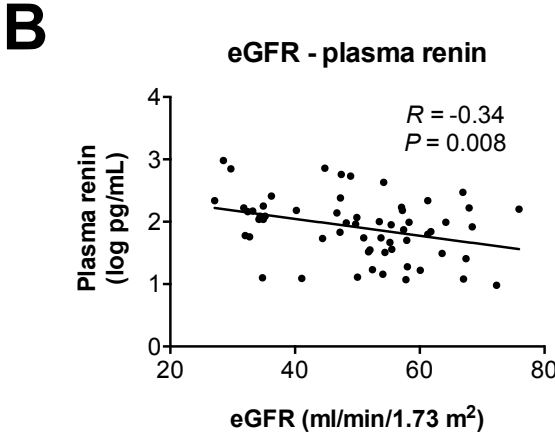
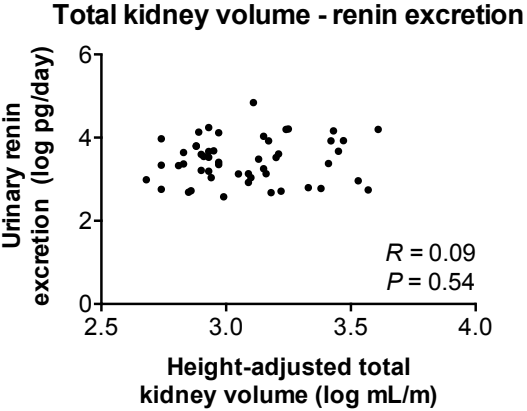
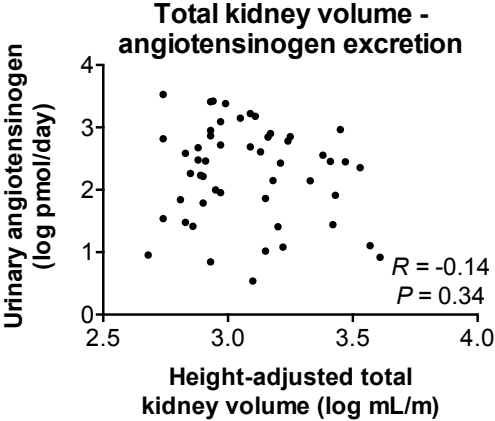
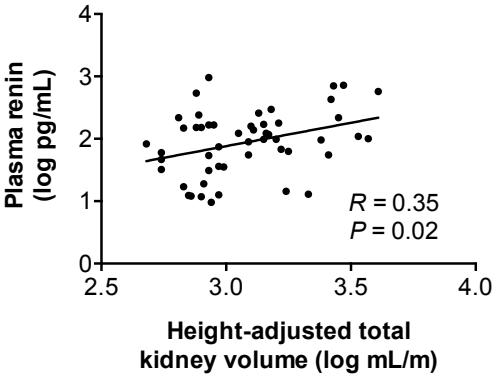
Urinary renin excretion



A Total kidney volume - albuminuria



Total kidney volume - plasma renin



Albumin AGT Prorenin Renin

